[0033] As mentioned, generally the subject RBC characterization methods measure the change in frequency of light waves, i.e., the change in frequency that light waves undergo when reflected by moving objects such as RBCs. Typically, skin is irradiated with coherent, single wavelength light which penetrates to a depth dependent on the wavelength of the light (the longer the wavelength, the deeper the penetration). A short distance away, light scattered back from the underlying tissue is detected by a broadband photodetector (the larger the distance between the source and detector, the deeper the tissue being observed). Light which has scattered back from immobile objects is the same frequency as the original illuminating beam. Light which is scattered back from moving objects, such as RBCs flowing in blood vessels, has a slightly shifted wavelength, with the shift dependant on the velocity of the moving objects. The shifted and unshifted light returning to the photodetector interacts in such a manner as to produce a low frequency (typically 0-20 kHz) oscillation or beat in the detected signal. The oscillating or AC component of the signal thus contains information about the velocity of flow of blood cells, while the average (DC) magnitude of the signal contains information about the total amount of light absorption and scattering in the tissue (which may correlate with the total amount of blood, both flowing and static, if the wavelength used is one where hemoglobin absorbs strongly).

[0034] Thus, a large average absorbance of light in ranges from about 450 nm to 600 nm or 850 nm to 950 nm indicates a high concentration of red blood cell-containing vessels, whether or not there was flow, where such a high concentration of red blood cell-containing vessels indicates a high concentration of arterioles, venuoles or capillaries. The AC signal is processed so that its power versus its frequency relationship is determined. The integral of this relationship between some lower and upper frequency bounds (e.g., 5 and 20 kHz) is determined, where the rate of flow increases as this integral increases. This integral is not completely linear with respect to flow, since higher frequencies are more sensitive to flow than lower ones. Therefore, outputs proportional to flow are employed, such as RBC flux. For example, formulas such as the formula

[0035] $$\text{RBC flux} = \left\langle \int_{f_l}^{f_u} fP(f)df - N \right\rangle / i^2$$

where ***f*** represents the shifted frequency, $\mathbf{f_l}$ and $\mathbf{f_u}$ represent the lower and upper cutoff frequencies, **P(f)** is the power at frequency f, **N** is a voltage offset and **i** is the mean photocurrent. RBC flux, as is known in the art (see for example Berardesca et al., *Bioengineering of the Skin: Cutaneous Blood Flow and Erythmea,* CRC Press, (1995)), may be used to generate outputs proportional to flow. The quantity or rather the magnitude of the RBC flux, as defined by the above-described formula, is substantially proportional to flow rate, where a high RBC flux corresponds to a high flow rate and a low RBC flux corresponds to a low RBC flux.

[0036] Accordingly, in the present invention, light at a wavelength in the range from about 400 nm to about 1200 nm, usually from about 450 nm to 800 nm is emitted from a light source such as a laser or the like and directed at the sample site, where such sources of light may be activated manually or automatically. The intensity of reflected light (the light reflected from red blood cells), and more specifically the change with time of the light, is measured and a value related to the character of the RBCs of the site, such as RBC flux, is determined. Such measurements may be fed into a microprocessor working under the control of a software program, where the microprocessor then determines the value related to the character of the RBCs of the site, such as RBC flux, which is proportional to the flow rate of a fluid in a vessel.

[0037] In one instance, the RBC characterization value, e.g., the RBC flux value or a statistically relevant value corresponding to the RBC flux value may be compared to a predetermined value, e.g., by means of a microprocessor. A comparison may then be made such that of the RBC value is above the predetermined value, the site is characterized as having a high flow rate and if the RBC value is below the predetermined value, the site is characterized as having a low flow rate. Alternatively, the best site (a highly appropriate site) amongst a plurality of potential sites tested may be determined by comparing RBC values of other tested sites.

[0038] Typically, RBC characterization is performed in about 1 to 180 seconds, usually in about 2 to 90 seconds and more usually in about 3 to 60 seconds.

II. SAMPLE TYPE CHARACTERIZATION

[0039] As described, the subject methods include sample type characterization, where such methods determine whether a site is capable of expressing or producing substantially arterial sample, substantially venous sample or substantially interstitial fluid. More specifically, when used in conjunction with the above described methods for characterizing flow, the particular sample type obtainable from a potential site can be characterized in regards to flow rates and sample type. In other words, a potential sampling site can be characterized as (1) high flow rate, arterial/capillary, (5a of Figure 1) (2) high flow rate, venous, (5b of Figure 1), (3) low flow rate, arterial/capillary or venous, (6b of Figure 1) or (4) low flow rate, interstitial fluid (6a of Figure 1). As noted above, the sample type characterization may be in addition to, or in place of, flow characterization, where the order of these may be changed or altered.

[0040] A variety of methods may be used to characterize the sample type obtainable from a potential sampling site, where pulse characterization and hemoglobin characterization are of particular interest. For example, if a high flow site is characterized as having a high pulse and/or a high oxygenated hemoglobin/deoxygenated hemoglobin ratio (where herein HbO represents oxygenated hemoglobin and Hb represents deoxygenated hemoglobin and HbO/Hb represents the ratio thereof), it is determined to be a site having substantially high flowing arterial sample (5a of Figure 1) and if a high flow site is characterized as having low pulse or low HbO/Hb ratio, it is determined to be a site having substantially high flowing venous sample (5b of Figure 1). Furthermore, if a low flow site is characterized as having a high total hemoglobin level or value it is determined to be a site of low flow arterial, capillary or venous sample (6b of Figure 1) and if a low flow site is characterized as having a low total hemoglobin level or value it is determined to be a site of interstitial fluid (6a of Figure 1). Thus, the subject invention provides methods that enable an individual to select a sampling site according to the amount or volume and/or type of sample obtainable from the site.

[0041] Any convenient method may be used to characterize the pulse and/or hemoglobin values or levels of a potential site, where RBC characterizations and hemoglobin characterizations (total hemoglobin and HbO/Hb ratio) are of particular interest. Each of these methods will now be described in greater detail.